PATENT
ATTORNEY DOCKET NO. 07340/044001

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant : Carson et al. Art Unit: 1806
Serial No.: 08/593,554 (Continuing Prosecution Application) Examiner: K. Hauda
Originally Filed: 1/30/96
Continuing Prosecution Application Filed: 3/8/98
Title : RECOMBINANT GENE EXPRESSION VECTORS AND METHODS FOR USE OF SAME TO ENHANCE THE IMMUNE RESPONSE OF A HOST TO AN ANTIGEN

Assistant Commissioner for Patents
Washington, DC 20231

DECLARATION OF EYAL RAZ, M.D. UNDER 37 C.F.R.§1.132
IN SUPPORT OF PRELIMINARY AMENDMENTS TO APPLICATION

I, Eyal Raz, M.D., declare as follows:

1. I am one of the named inventors in the above-titled Application and am an Assistant Professor at the University of California, San Diego School of Medicine. Since 1992, I have been a Principal Investigator specializing in immunology research at the Sam and Rose Stein Institute for Research on Aging, at the University of California, San Diego. I am also the Chief Scientific Officer for Dynavax Technologies Corporation, which develops nucleic acid vaccines and related products. The data provided in this Declaration were generated by me and by associates working under my direction. This Declaration supplements my previous declaration in this matter submitted in January, 1998.

Considered by Q.N. 9/25/00

Date of Deposit September 25, 1998
I hereby certify under 37 CFR 1.8(a) that this correspondence is being deposited with the United States Postal Service as first class mail with sufficient postage on the date indicated above and is addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231.

Germaine Sarda

GERMAINE SARDA

2. Hen egg ovalbumin (OVA) is an antigen which is widely used in studies of the mammalian immune response. It is a protein which, especially when delivered in alum, typically stimulates a robust, Th2 type immune response with inflammatory sequelae, including production of IgG*1* isotype antibodies with specificity for the antigen, IgE antibodies (including those with specificity for the antigen), and interleukin-4 cytokine. The same magnitude response is achieved on introduction of the OVA antigen together with an immunostimulatory oligonucleotide (ISS-ODN) whose immunostimulatory activity has been abolished (by methylation of the cytosine residue of the CpG core dinucleotide).

3. However, when OVA is introduced into an animal together with an active ISS-ODN of the invention, the immune response is predominantly one of the Th1 phenotype, as characterized by the production of IgG*2a* isotype antibodies specific for the antigen and "Th1-type" cytokines (e.g., IFN-γ), and production of Th1 promoting cytokines (e.g., IL-12). The magnitude of the Th1 type immune response in the presence of ISS-ODN is as much as 15 times the magnitude achieved through antigen immunization without ISS-ODN (e.g., IFN-γ levels following ISS-ODN-OVA conjugate co-administration are 15 times as high as the levels obtained following antigen administration without active ISS-ODN). This is true whether the OVA is administered as part of an ISS-ODN containing gene vaccine (i.e., a plasmid which codes for OVA and includes ISS-ODN in the backbone), is conjugated to a free ISS-ODN, or is co-administered concomittantly with the ISS-ODN. The most robust Th1-type immune responses are obtained through immunization with OVA-ISS-ODN conjugates. However, Th1 cytokine and antibody production in response to antigen expressed from a plasmid containing ISS-ODN in the backbone can be expected to be at least 2-3 times the magnitude obtained through immunization with antigen alone.

4. The Th2→Th1 phenotype switch described above is demonstrated by the data set forth in Figures 1-3 hereto. The data were obtained by immunizing groups of mice (4 mice/group) intradermally at day 0 and day 14 with: (a) 50 μg of OVA and 50 μg of pUC19 (a plasmid with two copies of the 5'-AACGTT-3' ISS-ODN in the backbone); (b) 50 μg of OVA and 50 μg of

methylated pUC19 (i.e., a plasmid whose ISS-ODN immunostimulatory activity has been abolished by substituting the CpG core dinucleotide with a GpG moiety); (c) 50 μg of OVA and 50 μg of a free, mutated ISS-ODN (DY1019, whose CpG mediated immunostimulatory activity has been abolished through substitution with GpG); (d) 50 μg of OVA-ISS-ODN conjugate (ISS-ODN=DY1018, which includes the 5'-AACGTT-3' core CpG sequence); or, (e) 50 μg pACB-OVA (a plasmid under the control of the CMV promoter which codes for OVA and includes two copies of the 5'-AACGTT-3' ISS-ODN in the backbone).

5. Figure 1 shows that the antigen-specific IgG1 (Th2 type antibodies) produced by all mice were produced at similar levels (in all groups except those which included the methylated, free ISS-ODN [DY1019], which produced higher titers). In contrast, Figure 2 shows that the levels of antigen specific IgG2a production by the mice were as much as 10 times, and at least 3 times, as high in the mice immunized with OVA and the ISS-ODN (DY1018) as compared to mice immunized with OVA alone or OVA and an inactivated mutant ISS-ODN (DY1019, with a GpG core dinucleotide). All IgG levels shown in the Figure are displayed in a log scale.

6. Figure 3 shows that IFNγ production (measured by pg/ml release of IFNγ from OVA stimulated CD4+ splenocytes obtained from the immunized mice) increased as much as 15 fold in the ISS-ODN/OVA immunized mice, as compared to mice immunized with OVA alone or OVA and an inactivated ISS-ODN (DY1019, with a GpG core dinucleotide).

7. Figures 4 and 5 show that IL-12 and IFNγ cytokine production (necessary for Th1 differentiation) from, respectively, mouse splenocytes (Figure 4) and macrophages (Figure 5), only occurs to a significant degree in the presence of active ISS-ODN, as compared to responses to inactivated ISS-ODN or a known immunostimulant, bacterial lipopolysaccharide (LPS). These data were obtained by culturing spleen cells ($2x10^6$ cells/well) or bone marrow derived macrophages ($2x10^5$ cells/well) with ISS-ODN (1μg/ml), methylated immune-inactived ISS-ODN (1 μg/ml), pUC19 (10 μg/ml), methylated immune-inactived pUC19 (10 μg/ml), LPS (5 μg/ml) or media alone. All experiments were performed without addition of antigen to elucidate

the uncombined effect of each compound on the target cells.

8. Further evidence that the Th1 phenotype produced in response to ISS-ODN is owing to their immunostimulatory activity and the core CpG motif is shown in Figure 6. Mice were immunized as described in paragraph 5 above with either the pCMV-LacZ plasmid (coding for β-galactosidase, with two copies of an ISS-ODN in the backbone), controls (without ISS-ODN) or mutated plasmid (wherein the CpG dinucleotide core of each ISS-ODN is substituted with a GpG dinucleotide) which suppresses immunostimulation. As shown in Figure 6, immunization with the mutated ISS-ODN abolished the Th1-stimulatory activity of the ISS-ODN: Compare the values for IgG2a production (Figure 6a); IFNγ production (Figure 6b) and antigen-specific CTL production (as indicated by % specific lysis of cells; Figure 6c) obtained for the active ISS-ODN containing pCMV-LacZ plasmid on one hand and the inactive ISS-ODN containing plasmid Z on the other hand (pZ/DY1019, pZ/DY1222 and pZ/cocktail).

9. Finally, others in the art have reached similar conclusions regarding the Th1-stimulatory activity of ISS-ODN administered in plasmids for use in gene vaccination. In this respect, see Klinman, *et al.*, *J.Immunol.*, 158:3635-3639 (1997) and the published summary of a lecture given by Dr. Arthur Krieg in Bethesda, Maryland on April 29, 1998 (Figure 7; addition of CpG motif containing oligonucleotides to an antigen-encoding plasmid results in enhancement of the host's subsequent response to the antigen HBsAg: the hepatitis B antigen; see in particular, the last paragraph of the lecture summary).

10. Taken together, these data conclusively demonstrate the role of ISS-ODN within a plasmids in the induction of a Th1 response to an encoded, conjugated or co-administered antigen.

All statements made herein are based on my own personal knowledge and with the knowledge that willful false statements and the like if made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or of any patent issuing thereon.

Dated: 9/1/98, 1998

Eyal Raz

Eyal Raz, M.D.

Executed at La Jolla, California

OFFICIAL
#20
12-13-00
P.2.

FACSIMILE TRANSMITTAL

BOZICEVIC, FIELD & FRANCIS LLP
200 Middlefield Road, Suite 200
Menlo Park, CA 94025
Telephone: (650) 327-3400
Facsimile Number: (650) 327-3231

OFFICIAL COMMUNICATION
DELIVER TO EXAMINER NGUYEN IMMEDIATELY

Date:	December 11, 2000
To:	U.S. Patent Office Examiner: Q. Nguyen Group: 1632
Facsimile No.	(703) 308-8724
From:	**Paula A. Borden**
Re:	U.S. Patent Application No. 09/265,191 for *Immune Stimulatory Compositions and Methods for Use of Same to Enhance the Immune Response of a Host to an Antigen* UC Reference: 92-296-1 MOFO Reference: 37788-20024.01 Our Reference: 6510-170CON2
Message:	Following is a Amendment in response to the Office Action dated September 26, 2000 in the above-captioned application. If you have any questions about this transmission please call me at (650) 833-7710. Thank you.

Total number of pages, including this cover sheet: **5**

****

Please contact **Mathew Otts** at (650) 327-3400 if you have any problems receiving this transmission.